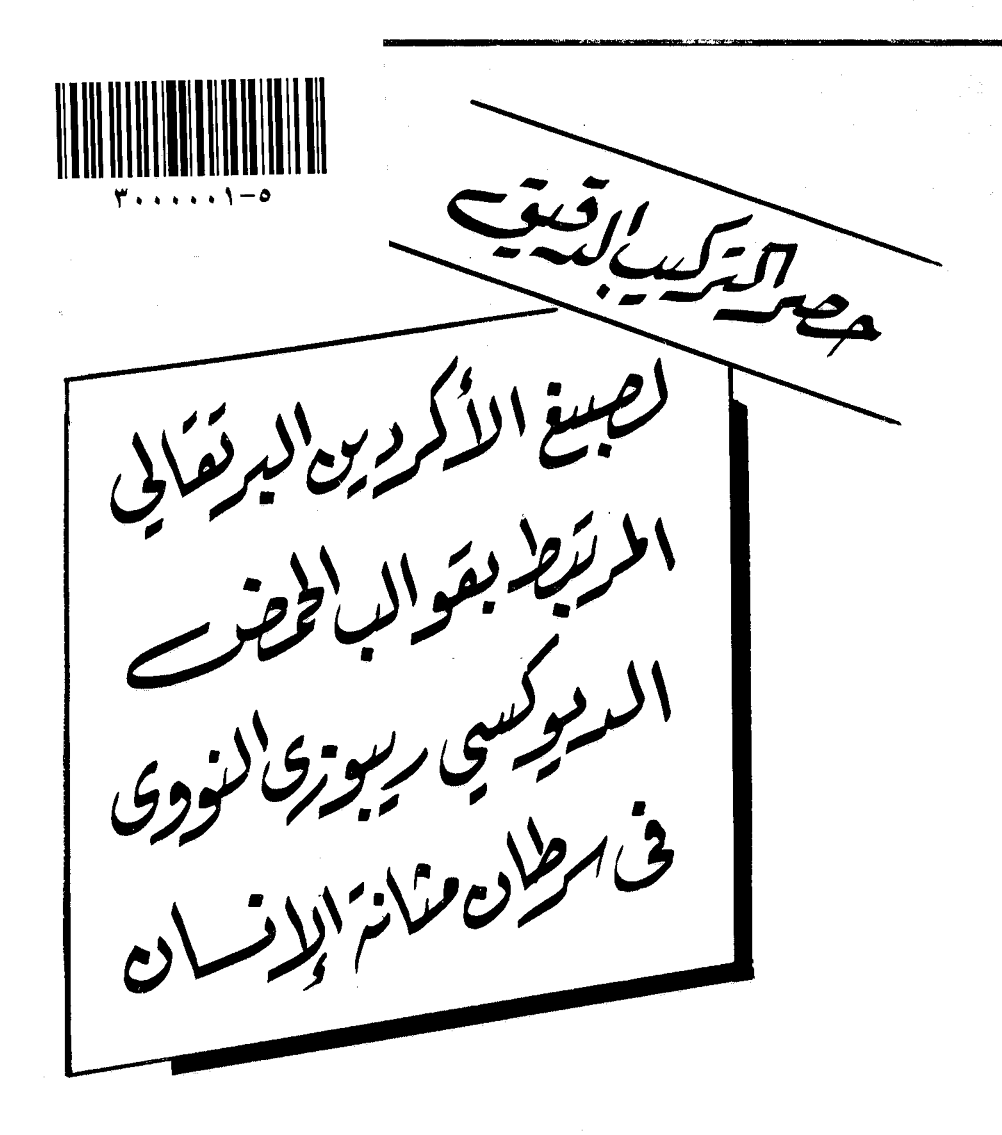


مجلة جامعة أم القرى

مجلة فصلية للبحوث العلمية المحكمة

السنة الأولى العدد الأول العام ١٤٠٩هـ



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« بسم الله الرحمن الرحيم »

حصر التركيب الدقيق لصبغ الاكردين البرتقالي المرتبط بقوالب الحمض الديوكسي ريبوزي النووي في سرطان مثانة الانسان

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كان الهدف الأساسي من هذه الدراسة التمييز بين الرتبة المنخفضة والرتبة المرتفعة لسرطان طلائية مثانة الانسان باستخدام صبغ الاكردين البرتقالي كطريقة كيميائية خلوية دقيقة .

وقد بينت الدراسة أن الزيادة المميزة لتفاعل الكروماتين بصبغ الاكردين البرتقالي في الرتبة المنخفضة لسرطان المثانة ترجع الى وجود حافز يعمل على مستوى قوالب الحمض الديوكسي ريبوزي النووي في الخلايا السرطانية وهذا الحافز كان موجودا أصلا في الخلايا السوية أثناء عملية التكوين الجنيني . وهذا الحافز يعمل على ازالة البروتين النووي الكابح لنشاط قوالب الحمض الديوكسي ريبوزي النووي .

وقد أظهرت الرتبة المرتفعة لسرطان المثانة خطوات متعاقبة من النضوج ، حيث أن حبيبات صبغ الاكردين تقل تدريجيا من الخلايا القاعدية الى الخلايا السطحية . وقد أمكن الافتراض أن جزءا ذا شأن من هذه الخلايا السرطانية المتقدمة لم تترك الدورة الانقسامية الا بفضل نقص نشاط قوالب حمض الديوكسي ريبوزي النووي .

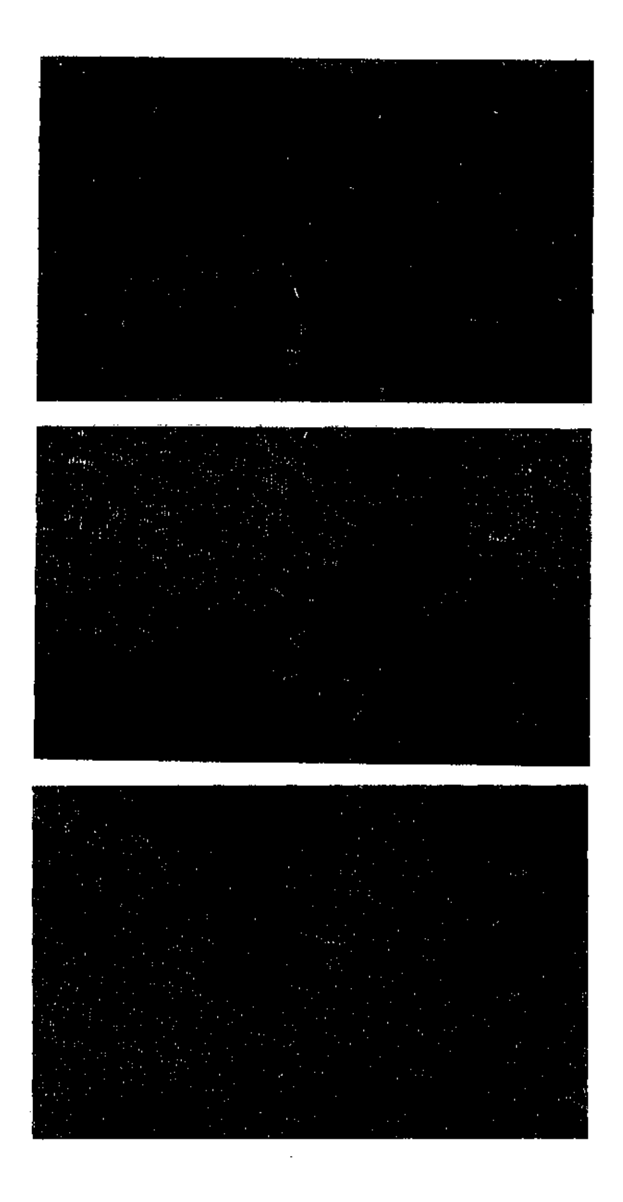


Fig. 2: Electron micrographs of high grade papilloma of human urothelium (a) Superficial cells, × 15,000 (b) Intermediate cells, × 9,000 (c) basal cells, × 15,000 Electron-dense reactions (arrows) is confined to the euchromatin.

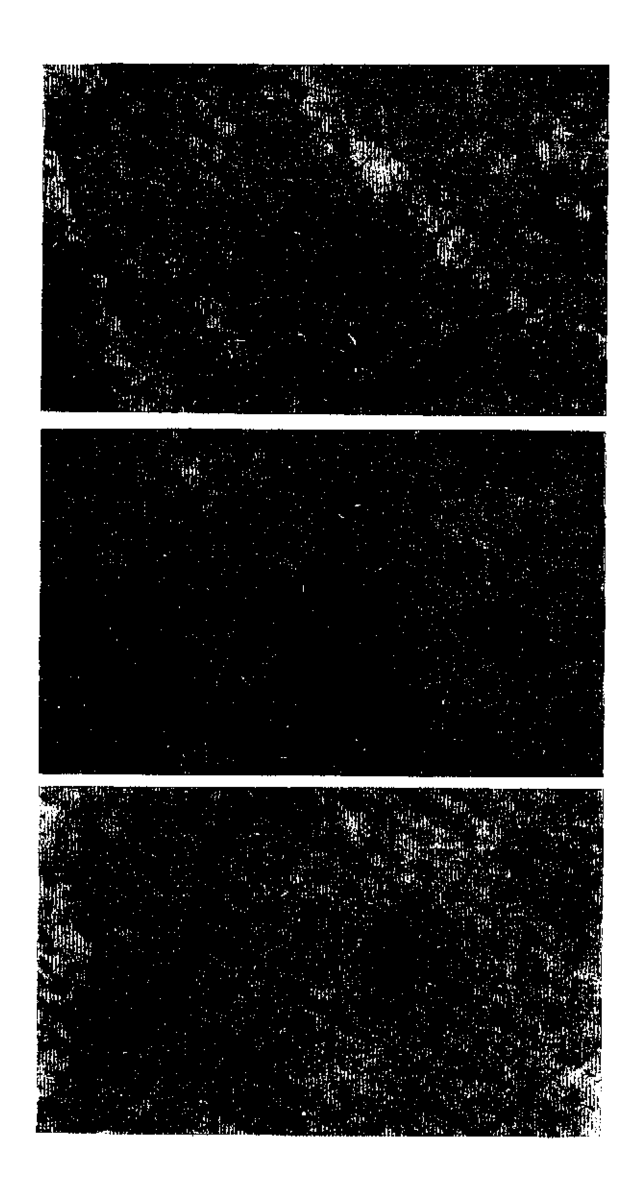


Fig. 1: Electron micrographs of low grade papilloma of human urothelium. (a) Superficial cells, × 9,000 (b) Intermediate cells, × 15,000 (c) basal cells, × 15,000. Electron-dense reactions (arrows) is confined to the euchromatin.

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include the cells of the high grade papillary tumours of the present material, which was found to have less DNA template activity than the normal counterpart.

Finally, study of the DNA template activites in bladder tumours is not only an important research tool, but also appears to have clinical application in patients who have tumour and resist endoscopic therapy, radiotherapy or chemotherapy. A biopsy specimen of such tumour could be processed for AO chromatin interaction and if there is a high DNA template activity in all the cell layers, this would be considered as low grade tumour. However, if there is a low DNA template activity and a decline of the probes number from the base to the surface, the tumour would be considered as high grade tumour.

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Discussion

In the Low grades papillary tumours, the cells were found to have high amount of DNA template activity. Increasing derepression of DNA templates was noted if neoplastic cells were assayed for the diversity of RNA species being synthesized as the neoplasm progressed first to a benign nodule, then to a spontaneous neoplasm, and finally to a transplantable highy malignant neoplasm (Turkington, 1971). Also, Veneema et al. (1971) observed that well differentiated tumours had unexpectedly high rate of nucleic acid synthesis.

During the course of embryogenesis in the developing liver, a progressive decrease in the diversity of RNA species being synthesized was observed, but when the adult liver was induced to regenerate following partial hepatectomy, derepression of previously repressed DNA templates again allowed the reappearance of those RNA species characteristic of the embryonic state (Church and Mc Carthy,1967). Such a derepression of normally repressed DNA template may account for the reappearance within adult human neoplasms of cell surface antigens characteristic of normal fetal life (Gold, 1971). A quite similar reappearance of normal fetal antigens has been observed in both chemically induced and virus induced neoplasms in experimental animals (Herstein and Frenster, 1972).

In the high grade carcinoma, the DNA template activities were found to decline as cell differentiation progressed, but that decrease was less than that found in the normal urothelium. A similar decrease has been observed during nuclear blebbing maturation of human acute Leukemia blast cells (Ahearn and Trujillo, 1973) and during the nuclear blebbing (Frenster et al. 1975) and maturation (Frenster et al., 1976) of neoplastic Reed-sternberg cells in the lymph nodes of untreated patients with Hodgkin's disease. It has been suggested that during the course of neoplastic cell survival a signifiant fraction of advanced neoplastic cells leave the proliverastive cycle by virtue of decreasing their DNA template activity (Peckham and Cooper, 1969; Saunders and Mauer, 1969; Ahearn and Trujillo, 1973), and that might

Results

As previously shown in other systems (Frenster, 1971; Lehmann and Slavkin, 1976) incubation with AO and subsequent digestion with DNase resulted in electron-dense reaction products within nuclei of normal and tumour cells of human urothelium. The AO chromatin reaction products were specifically localized over the euchromatin portion of the nucleus. They were not found in the heterochromatin, cytoplasm or associated with extracellular matrix components (Figs. 1 and 2).

Observations on the normal human urothelium were listed in a previous report (Ismail, in press). It was found that the number of AO probes decline as normal cell differentiation progressed. Also, the number of the probes decline but to a lesser degree in high grade tumour (table 1 and fig. 2 a,b,c). However, in low grade tumour the situation is different and it seems to be constant from the base to the surface (table 1 and fig. 1 a,b,c).

In Low grade tumours the number of AO chromatin interaction was found to be significantly higher than that of normal urothelium especially in the intermediate and superficial cells (table 1). However, in high grade tumour the number of the probes in the basal and intermediate cells was found to be significantly less than those of normal urothelium (table 1).

Table (1) : The mean probe count of human papillary tumour and the normal counterpart. The standard error of each value is indicated.

Cell type	Normal	Low grade		High grade
Superficial	-	96.88±8.76		18.45±2.59
			P<0.001	
Intermediate	53.15±5.82	88.59±13.53		30.04±3.88
	P<0.001		P<0.001	
		P<0.001		
Basal	79.10±5.82	95.65.±7.75		38.75±6.51
	P<0.01		P<0.001	
		P<0.001		

grading of bladder tumours is consistent and reproducable, in the first place to ascertain that the right patient gets the right treatment (Sjølin et al., 1976). Moreover, the comparison of the clinical results of different institutions is invalidated by a great variation in grading results (Ooms et al., 1981).

In this paper, it is considered interesting to study the AO binding sites within nuclei of human bladder carcinomas in order to find out if any variation occurs in low and high grades.

Material and Methods

The human papillary tumour biopsies were obtained with Storz forceps, mainly from elderly males who had no other symptoms suggestive of infection or urinary obstruction, and the samples were taken before any form of treatment was initiated. The samples were initially checked by light microscopy. The light microscope examination and tumour grading were carried out according to the WHO grading system.

The method described by Frenster (1971) was applied. After fixation in 5% glutaraldehyde in medium 199 for 2 hours, the fixed tissue were treated with 10^{-3}M acridine orange in medium 199 for 1 hour. The samples were next washed three times in medium 199 and incubated at 37°C for 30 minutes in Eagle's minimum essential medium containing 1.0 mg/ml pancreatic DNase I. The incubated samples were then prepared for electron microscopy by being postfixed for 1 hour in 1% osmium tetroxide, dehydrated with graded concentration of ethanol and propylene oxide, and embedded in Epon. Thin sections were cut with an LKB ultramicrotome, stained with 5% uranyl acetate for 30 minutes, and examined at 80 KV in an AEI-BM6 electron microscope.

Statistical analysis: A random sample of five blocks from each of the samples to be analysed was used to count within each cell the electon-dense granules. Statistical comparisons between cells of the normal urothelium, the low and high grades of papillary carcinomas were made using the student t-test.

Ultrastructural Localization of Acridine Orange Binding to DNA Template in Human Bladder Cancer.

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Abstract

The primary object of the present study was to distinguish the low grade tumour from the high grade tumour of human urothelium, using the acridine orange (AO) as ultracytochemical probe.

In the present study, the increased interaction of AO chromatin observed in the low grade tumour was attributed to the presence of derepressors in the neoplastic cells which are normally found during embryogenesis. However, the high grade tumour showed definite sequences of cell maturation, where the number of AO probes declines from the base to the surface. It has been suggested that a considerable fraction of advanced neoplastic cells leaves the proliverative cycle by virtue of decreasing their DNA template activity.

Introduction

Broders (1922) introduced a system for grading of bladder tumours. Bergkvist et al. (1965) and Pugh (1973) formulated their tumour grading in more detail. Also, in 1973, the WHO divided the urothelial tumours into three grades . Anaplastic carcinomas formed a separte group in this grading system. However, these three grading systems are basically quite similar in that the grades I, II and III in the three grading systems do not differ significantly, and that the grade IV tumours in Bergkvists' classification are designated as anaplastic carcinomas in the WHO system.

In clinical practice, grade I and II tumours (Low grade tumours) are treated conservatively with transurethral resection or with local chemotherapeutical application, Whereas the high grade tumours (grade III and over) are treated more aggressively. It is clear that it would be very desirable if the